CERTIFICATE OF MAILING
37 C.F.R. 1.8

I hereby certify that this correspondence is being deposited with the U.S. Postal Service with sufficient postage as First Class Mail in an envelope addressed to: Commissioner for Patents, Washington, DC 20231, on the date below:

August 7, 2002 — Date
Charles P. Landrum

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Ruth A. Gjerset

Serial No.: 09/556,440

Filed: April 24, 2000

For: DOWN-REGULATED OF DNA REPAIR TO ENHANCE SENSITIVITY TO P53-MEDIATED SUPPRESSION

Group Art Unit: 1642

Examiner: B. Brumback

Atty. Dkt. No.: INRP:032—2/SLH

DECLARATION OF DEBORAH R. WILSON, PH.D, UNDER 37 C.F.R. 1.132

Commissioner of Patents
Washington, D.C. 20231

I, Deborah R. Wilson, Ph.D, declare that:

1. I am the Associate Vice President of Clinical Research at Introgen Therapeutics, Inc. ("Introgen"), assignee of the above-captioned application. I have been employed at Introgen for 7 years and was recently named Associate Vice President. My responsibilities as Associate Vice President of Clinical Research at Introgen include clinical science, pharmacokinetics, and drug safety. I am a citizen of the United States of America, and I reside at 11022 Silkwood, Houston, Texas 77031.

2. I understand that the Patent and Trademark Office has rejected claims in the above-referenced case as lacking enablement, based on reasons related to the lack of success of gene therapy.

3. Introgen and its collaborators have been conducting research and development of an Ad-p53 composition for the treatment of cancer for at least 10 years. Introgen's research and development has progressed to the point where its Ad-p53 composition, INGN 201 (Introgen's Advexin® adenovirus p53 product), which is disclosed in the present application, is involved in a number of clinical trials for head and neck cancer, lung cancer, breast cancer, esophageal cancer, glioma, prostate cancer, advanced solid tumors, bladder cancer, and ovarian cancer. *See* Table of Adenovirus-p53 Clinical Trials (Exhibit 1). INGN 201 is in phase III clinical trials for head and neck cancer. Phase II clinical trials are underway or have been completed for head and neck cancer, esophageal cancer, breast cancer, and non-small cell lung carcinoma. INGN 201 was used or has been approved for phase I clinical trials for lung cancer, breast cancer, liver cancer, glioma, prostate cancer, head and neck cancer, bladder cancer, ovarian cancer, colorectal cancer, malignant ascites, and solid tumors from a variety of origins.

4. Several clinical trials have been conducted for various cancers including ovarian cancer, lung cancer, bladder cancer, and metastatic colorectal cancer using a different Ad-p53 construct from another company, Schering Plough.[1]

5. The clinical trials discussed in paragraphs 3 and 4 involved or will involve a variety of administrations of Ad-p53 constructs. Administrations include: intraperitoneal,

[1] *See, e.g.*, Barnard (2000); Horowitz (1999); Kuball *et al.* (2002); Schuler *et al.* (2001) and the reference of Wills *et al.*, which provides the details regarding the structure of the SCH 58500 Ad-p53 construct, which lacks protein IX. (Exhibit 2)

intravenous, intravesical, intratumoral, intramucosal injection, oral rinse, and broncho-alveolar lavage.

6. I anticipate Introgen will proceed with other clinical trials in the future involving adenovirus-p53 constructs, given the success I have observed in the ongoing or previous clinical trials with Introgen's product, INGN 201.

7. I declare that all statements made herein of my own knowledge are true, and that all statements of my own belief are believed to be true, and further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under § 1001 of title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this patent, and any reexamination certificate issuing thereon.

6 August 2002
Date

Deborah R. Wilson, Ph.D.

EXHIBIT 1

Table of Adenovirus-p53 Clinical Trials (as of February 2002)

Treatment	Cancer	Admin	Clinical Stage	Status/Result
INGN 201	SCCHN (T301)	Intratumoral	III	Ongoing
INGN 201	SCCHN (T302)	Intratumoral (with chemotherapy)	III	Ongoing
INGN 201	NSCLC	Intratumoral (with radiation therapy)	II	Combination INGN 201 and radiation therapy appears more effective than radiation alone
INGN 201	SCCHN (T207)	Intratumoral	II	Safe
INGN201	Locally advanced primary breast	Intratumoral (with chemotherapy)	II	Study has been initiated
INGN 201	Esophageal	Intratumoral	II	Ongoing
INGN 201	SCCHN (T201)	Intratumoral	II	Safe; demonstrated clinical activity
INGN 201	SCCHN (T202)	Intratumoral (lower dose)	II	Safe; trend towards shorter survival than T201
INGN 201	Ovarian	Intraperitoneal	I	Transgene expression observed and increased expression of downstream marker; well-tolerated
INGN 201	Ovarian	Intraperitoneal (laparoscopy)	I	Well-tolerated; potentially useful clinical response
INGN 201	Bladder	Intravesical	I	Transgene expression observed; safe; ongoing

INGN 201	Advanced solid tumors (colon, breast, prostate, sarcoma, NSCLC, H&N)	Intravenous	I	Well tolerated at doses up to $1x10^{12}$ vp; accrual is ongoing to further determine MTD; evaluation of p53 expression is pending
INGN 201	SCCHN	Intratumoral (with and without tumor resection)	I	Transgene expression and expression of downstream targets observed; safe; potentially useful clinical response
INGN 201	NSCLC	Intratumoral	I	Transgene expression and apoptosis observed; safe; potentially useful clinical response
INGN 201	NSCLC	Intratumoral (with cisplatin)	I	Expression observed; well tolerated; potentially useful clinical response
INGN 201	Prostate	Intratumoral (INGN 201 treatment prior to tumor resection)	I	Transgene expression and apoptosis demonstrated; safe
INGN 201	Glioma	Intratumoral and intracranial (stereotactic injection intratumorally, followed by tumor resection, followed by injection into tumor bed	I	Expression observed; safe; apoptosis observed; ongoing
INGN 201	Hepatocellular Carcinoma	Intratumoral	I	Study closed; 1 patient treated

INGN 201	Breast	Intratumoral (with chemotherapy)	I	Study closed; 2 patients treated
INGN 201	Bronchioloalveolar lung carcinoma	Broncho-alveolar lavage	I	Safe; potentially useful clinical response; ongoing
INGN 201	Malignant ascites	Intraperitoneal	I	Study closed; 1 patient treated
INGN 201	Colorectal	Intratumoral	I	Study closed; 6 patients treated; expression of downstream markers demonstrated
INGN 201	Lung	Intratumoral (with and without cisplatin)	I	Ongoing
INGN 201	Oral dysplasia (premalignant)	Intramucosal injection; oral rinse		Not started
SCH 58500	Ovarian	Intraperitoneal (with chemotherapy)	II/III	Reported closed
SCH 58500	Lung	Intratumoral (with chemotherapy)	II	Transgene expression observed; well-tolerated; enhanced local effects suggested with certain chemotherapies
SCH 58500	Ovarian	Intraperitoneal (with chemotherapy)	I/II	Well tolerated; expression observed; prolonged patient survival

SCH 58500	Lung	Intratumoral	I	Transgene expression and expression of downstream target observed; safe; transient tumor growth control
SCH 58500	Bladder	Intratumoral or intravesical (with transduction enhancer)	I	Transgene expression and expression of downstream marker demonstrated after intravesical instillation

ELSEVIER

Journal of Photochemistry and Photobiology B: Biology 63 (2001) 162–170

Journal of Photochemistry and Photobiology B:Biology

www.elsevier.com/locate/jphotobiol

DNA repair inhibition and cancer therapy

Niall M.B. Martin*

KuDOS Pharmaceuticals Ltd, 327 Cambridge Science Park, Milton Road, Cambridge, Cambs. CB4 4WG, UK

Received 6 August 2001; accepted 13 August 2001

Abstract

The DNA repair process in mammalian cells is a multi-pathway mechanism that protects cells from the plethora of DNA damaging agents that are known to attack nuclear DNA. Moreover, the majority of current anticancer therapies (e.g. ionising radiation and chemotoxic therapies) rely on this ability to create DNA lesions, leading to apoptosis/cell death. A cells natural ability to repair such DNA damage is a major cause of resistance to these existing antitumour agents. It seems logical, therefore, that by modulating these repair mechanisms, greater killing effect to anticancer agents would occur. Experimental data support this, either through knockout studies or by the use of pharmacological inhibitors which target some of the key regulatory proteins involved in the DNA repair process. Several of these key DNA repair proteins which are actively under investigation as novel sites for intervention in cancer biology are discussed.

Keywords: DNA repair inhibition; Cancer therapy

1. Introduction

Cancer within the developed countries is on the increase, despite an improved understanding of the causes of the disease. Approximately 70% of all these cancer patients will receive radiation therapy, while 50% of all newly diagnosed cancer patients will receive some form of chemotherapy [1]. The challenge is therefore two-fold: to identify new "less-toxic" anti-cancer drugs from those of the conventional radiation and chemotherapy treatments, or to improve these existing therapies, so that they become far more effective in the battle against cancer. Cellular DNA repair may provide a means of achieving some of these aims.

Mechanistically, ionizing radiation (IR) and chemotherapeutic drugs interfere with cell division by introducing DNA lesions. While both therapies tend to be more toxic to rapidly dividing cancer cells, they are often not sufficiently specific and can affect normally growing cells [2]. As a result, patients often experience debilitating side effects [3]. For chemotherapies, such side effects can limit the effectiveness of the therapy, because the clinician must avoid exceeding the maximum tolerated dose. It may thus not be possible to administer sufficiently high doses of chemotherapeutic drugs to overcome the natural resistance of the cancer cells. Causes of resistance to the effects of irradiation (ionizing radiation) and chemotherapeutic drugs often stems from the tumour's ability to overcome their damaging actions. As such, DNA repair pathways are central to this process [4]. In fact, DNA repair is a double-edged sword in tumour biology. The whole repair machinery is vitally important to the maintenance of genomic integrity, viability and growth of normal cells. However, the introduction of mutations and loss of proper functioning repair mechanisms in their own right may lead to the development of neoplastic cells and cancer. It is these same repair pathways that confer resistance to the cancer killing effects of the most established therapies, IR and chemotherapeutics (for review, see Ref. [4]). Therefore, it is necessary to understand these DNA repair pathways in order to be able to use them to our advantage as potential sites for developing novel anti-cancer therapies. Certainly, by targeting some of the key proteins in the DNA repair processes it is hoped that, in combination with the existing therapies of ionizing radiation and chemotoxic drugs, we can improve the effectiveness of treatments.

2. DNA repair in tumour development

Tumorigenesis is a multi-step process that reflects genetic alterations, which drive the progressive transforma-

*Fax: +44-1223-719-719.
E-mail address: nmartin@kudospharma.co.uk (N.M.B. Martin).

1011-1344/01/$ - see front matter
PII: S1011-1344(01)00213-5

tion of normal cells into highly malignant derivatives. This "genomic instability" is fuelled by DNA damage and by errors introduced by the DNA replication process. A large diversity of factors have been identified that are either endogenous in source (e.g. by products from metabolism) or exogenous (e.g. sunlight, ionizing radiation, genotoxic chemicals and tobacco smoke) which may lead to an array of different DNA lesion types [5–8]. In view of the plethora of DNA damaging agents that can cause various lesion types, no single repair process seems to be able to cope with all kinds of damage. Instead, a number of sophisticated and interactive DNA-repair systems have evolved to cover most of the insults inflicted on the cells vital genetic information (Fig. 1).

At least four main, partly overlapping, damage repair pathways are known to operate in mammalian cells [4]. Two pathways, which deal with a number of DNA helix-distorting lesions and single strand breaks, are the nucleotide-excision repair (NER) and base-excision repair (BER) pathways. Whereas damage that is severe enough to cause both strands of the DNA to break is either dealt with by the non-homologous end-joining pathway (NHEJ) and/or the less error-prone homologous recombination pathways (HR) [8].

It is interesting to note that by targeting an individual pathway in the DNA repair machinery you will inadvertently sensitise cells to the killing effects of therapies which introduce differing levels of lesions into the DNA. For example, at low dose levels, most of the DNA damage caused by ionising radiation can be repaired. However, inhibition of DNA repair permits the conversion of this otherwise repairable damage into irreparable damage and can thus lead to more apoptosis. So, it remains to be seen which repair pathways afford the greatest effects on the therapeutic indexes of conventional genotoxic agents.

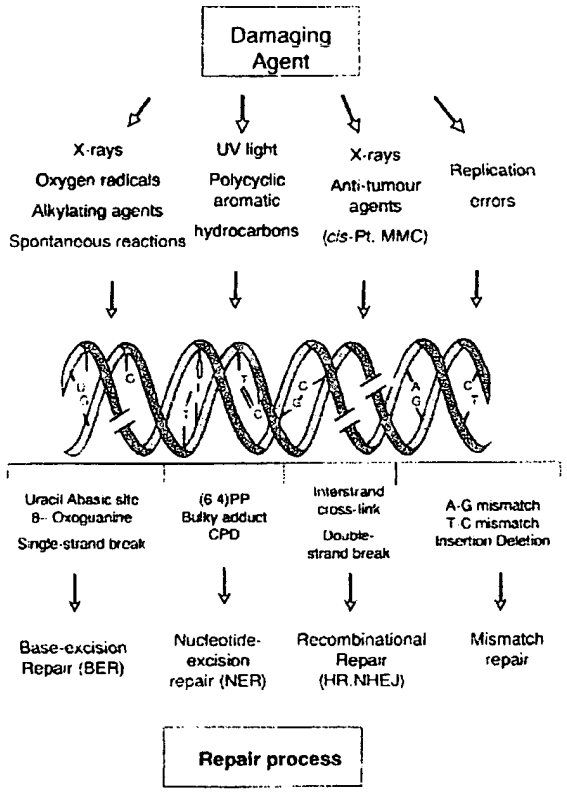


Fig. 1. DNA damage and the repair mechanisms involved. A variety of damaging agents, either exogenous or endogenous in source, can induce different DNA damage lesion types. Consequently, the DNA damage repair mechanisms are activated depending on the lesions involved. Abbreviations: *cis*-Pt and MMC, cisplatin and Mitomycin C; (6 4)PP and CPD, 6-4 photoproduct and cyclobutane pyrimidine dimer; BER and NER, base-excision and nucleotide-excision repair; HR and NHEJ, homologous recombination and non-homologous end joining.

3. Excision repair and single stranded break repair

Nucleotide-excision is the principal mechanism involved in the removal of DNA lesions that are generated by the UV effects of sunlight. These lesions (cyclobutane-pyrimidine dimers (CPDs) or pyrimidine-6-4 pyrimidone photoproducts) cause a distortion of the DNA [9–11], leading to abnormal replication. The importance of the ubiquitous NER process in maintaining genomic DNA integrity from the damaging effects of UV radiation is indicated by the fact that defects in certain repair genes of this pathway lead to sun-sensitive and cancer-prone genetic disorders in humans, such as xeroderma pigmentosis (XP) and Cockayne's syndrome (CS) [12,13].

However, it is the base-excision repair (BER) pathway, on the other hand, which is critical for single strand break (SSB) repair. The BER differs from that of NER in that it also plays a role in the removal of altered chemical bases (e.g. abasic sites, uracil incorporation, and methylation sites), as well as the repair of SSB. The action of X-rays, the production of oxygen radicals and the effects of alkylating agents, such as Mitomycin C and cisplatin, are the principal causes of SSB damage in genomic DNA. As a consequence of this central role in DNA damage repair, as induced by genotoxic agents, the BER pathway presents an ideal opportunity for pharmacological intervention, as adjuncts to conventional therapies [14]. One of the key initiators for the detection and signalling of SSB repair, which represents a major target of interest in tumour biology, is the nuclear enzyme, poly(ADP-ribose) polymerase-1 or PARP-1 [15].

4. Poly(ADP-ribose) polymerase and related proteins

Although no human disorders are known where BER deficiencies have been identified, the importance of the BER process, in particular the participation of PARP-1, has

been established through various "knockout" animal and cellular studies [16,17]. The dependence of PARP-1 activity on DNA breaks has led to a considerable amount of work towards understanding the role of PARP-1 in DNA repair. $PARP^{-/-}$ mice are hypersensitive to high doses of whole body γ-irradiation and cell-lines derived from such mice also show an increased susceptibility to radiation and alkylating agent [18,19]. This has been confirmed through the use of PARP inhibitors, which showed that inhibition of PARP-1 activity results in increased sensitivity to chemo- and/or radiotherapies (for review, see Ref. [20]).

The first identified PARP-1 inhibitors were based around the nicotinamide moiety of NAD^+, where nicotinamide (Fig. 2) and 5-methylnicotinamide were shown to function as competitors of NAD^+. Subsequently, nicotinamide isosteres, such as benzamide, were synthesised and showed weak PARP-1 inhibition. In an attempt to find more specific inhibitors, 3-aminobenzamide (Fig. 2) was identified [21]. Until recently, 3-AB has been the "benchmark" inhibitor for PARP-1; however, as with most of these early compounds, it lacked sufficient potency to be of real clinical use. The advent of high throughput screening (HTS) and the release of the crystal structure of the catalytic domain for chicken PARP, with nicotinamide-analogue inhibitors incorporated [22], has generated far more potent, and perhaps more selective, inhibitors [20,23–25]. A new generation of PARP inhibitors, based upon the NAD^+ moiety and early inhibitor studies, are some of the most potent, selective and soluble PARP-1 inhibitors known to date (Fig. 2).

It is now well established that the inhibition of PARP-1 potentiates the killing actions of several antitumour agents. For example, 3-AB has been shown to decrease cell survival of various cell-lines to the actions of bleomycin, 1-methyl-3-nitroso-guanidine (MNNG), methyl methanesulfonate (MMS), γ-radiation and X-rays (for review, see Ref. [26]). The PARP-1 inhibitor, 4-amino-1,8-naphthalimide, also significantly enhanced the effect of γ-irradiation cytotoxicity on several tumour cell-lines [27]. Generally, levels of kill, normally expressed as a potentiation factor at 50% inhibited growth relative to controls (PF_{50} — ratio of control IC_{50} vs. sample IC_{50}), have shown that quite significant enhancements of activity by PARP-1 inhibitors over and above antitumour agents alone are achievable [28]. Interestingly, the relative PF_{50} values very much depend on the cell-line that is under inves-

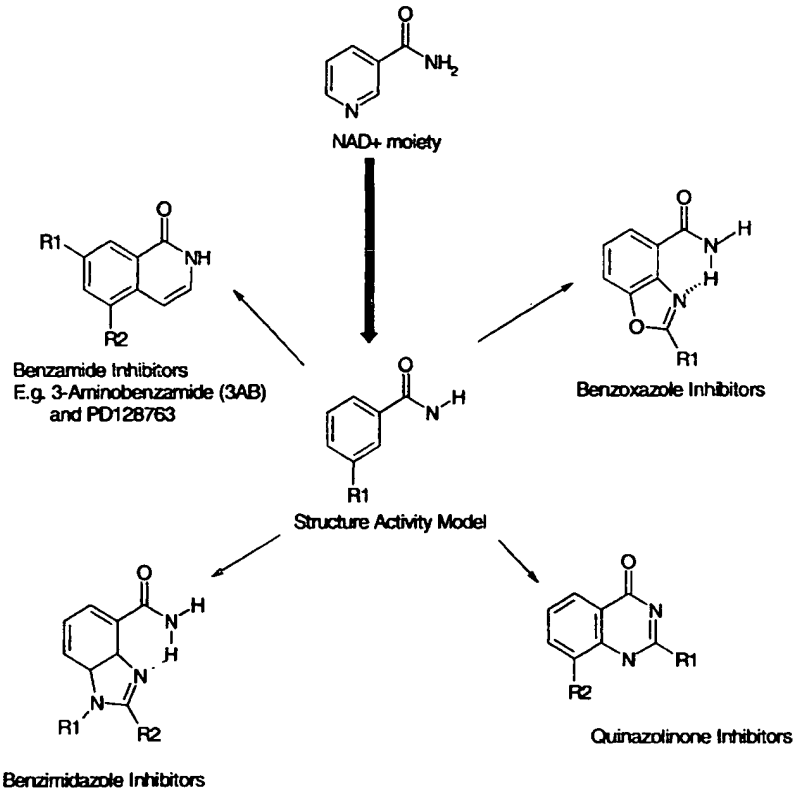


Fig. 2. PARP-1 inhibitors derived from the nicotinamide core structure. Several series of potent PARP inhibitors have been developed from the original binding structure of nicotinamide (NAD^+). Some of the more potent structures, such as the benzimidazoles, are in or near to preclinical development (for review, see Ref. [20]).

tigation, presumably indicating the resistance of some tumour cells to PARP-1 inhibition, an important factor when addressing the clinical direction of such drugs [28].

Moreover, in vivo studies have very much confirmed the in vitro data, where, for example, the use of 3-AB has demonstrated that PARP-1 inhibition will enhance the antitumour activity of several drugs, including bleomycin [29], cisplatin [30], chlorambucil [31] and cyclophosphamide [32]. While in vivo treatment of mice bearing SCC7 sarcomas, where the PARP-1 inhibitor PD128763 was used in combination with 2.5 G_y of X-irradiation, caused tumour regressions and significant tumour growth delay [33]. This treatment regime resulted in more than a three-fold increase of the therapeutic effect of X-rays alone [33]. Nevertheless, despite significant work in this area, no PARP-1 inhibitors have so far reached clinical trials, although some may be in late stage preclinical testing.

One important issue, however, that is still outstanding, even with the new generation of PARP inhibitors, is selectivity amongst the ever expanding family of PARP proteins. For example, PARP-2 works in tandem with PARP-1 and plays a role in DNA damage repair similar to that of PARP-1, although PARP-1 accounts for the majority of the cellular activity seen [34]. As such, NAD^+ competitive inhibitors are known to inhibit PARP-2 potentially to the same affinity as PARP-1 (KuDOS, unpublished data). This may not pose such a problem if this inhibition was restricted solely to PARP-2. However, the catalytic domain is reasonably well conserved across the PARP "family" [35], and so it remains uncertain what effects catalytic site targeted inhibitors will exhibit on the other PARP family members such as VPARP or tankyrase. Furthermore, the long-term effects of PARP-1 inhibition are unknown. In $PARP^{-/-}$ mice, despite a normal phenotype [18], it has been demonstrated that, on exposure to carcinogens such as *N*-nitrosobis (2-hydroxypropyl)amine (BHP), the incidence of induced tumours in multiple organs is significantly increased from those seen in non-mutant treated mice [36]. Yet it looks, on balance, that the benefits of PARP-1 inhibition as an adjunct therapy with IR or chemotoxic drugs, over an "acute" treatment period of weeks, will outweigh any possible risks.

Interestingly, PARP-1 is known to bind to p53 protein and form a tight complex with wild type as well as mutant forms of the p53 tumour suppressor protein [37,38]. ADP-ribose polymers that target specific binding sites in the p53 protein regulate the binding properties of p53 [38]. This interaction has become more evident with the recent use of double null mutant mice, where it has been demonstrated that tumour-free survival of the double null mutant $PARP^{-/-}p53^{-/-}$ mice increased by 50% as compared with that of $PARP^{+/+}p53^{-/-}$ animals [39]. These studies suggest that, in specific tumours expressing functional p53, PARP-1 inhibition may sensitise tumour cells to irradiation, while, in addition, in a p53 mutant background, PARP-1 inhibition could improve the clinical outcome by retarding the growth of tumours deficient in functional p53 [39].

5. Double stranded break repair

Major damage to DNA, which results in DNA double strand breaks (DSB), is in general lethal to cells. Such lethal damage can arise from a variety of factors, including X-rays, ionizing radiation and the use of genotoxic-chemotherapeutic drugs, for example alkylating agents such as cisplatin, Mitomycin C, MNNG and topoisomerase II inhibitors like etoposide (for review, see Ref. [5]). Moreover, free radicals (i.e. generated oxygen species or peroxynitrites) as well as genomic replication involving a single strand break can also lead to the production of DSB lesions [4].

In humans, DSB repair involves two principal but distinct routes, the homologous recombination (HR) pathway and non-homologous end-joining pathway (NHEJ). Both are multi-factorial transduction cascades that can halt either the cell-cycle machinery and/or recruit repair factors to the damaged site on the DNA [40,41]. Until recently it was thought that the NHEJ pathway was the primary mechanism for repairing DSBs in mammalian cells, based upon the fact that rodent-cell mutants for several genes involved in NHEJ, including Ku70, Ku86, DNA-PK, Ligase4 and XRCC4, all showed increasing sensitivity to IR, resulting in cell killing and induction of chromosomal aberrations [41,42]. A number of nuclear proteins have been shown to have a role in the mechanism of DSB repair. Individual roles or functions of these proteins are actively under investigation, and their interactions with other repair proteins and mechanisms are being elucidated. Some of the key players in the NHEJ pathway identified to date are discussed below.

6. ATM, ATR and DNA-PKcs related kinases

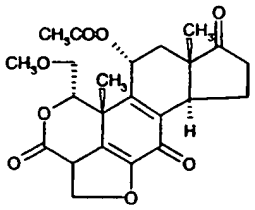
The detection and signalling to the NHEJ pathway involves a number of members of the phosphatidylinositol 3-kinase like kinase (PIKK) family. These serine/threonine kinases include ATM (*ataxia-telengiectasia* (A-T) mutated), its related protein ATR (A-T related protein) and the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) [43]. These enzymes function by binding to the ends of DNA generated by double strand breaks, where they become activated and initiate DNA repair.

The importance of ATM as a central controlling factor following DNA damage involving DSB is shown by the number of downstream targets that it phosphorylates, including p53, Brca1, Mdm2 and the cell cycle checkpoint control kinases Chk1 and Chk2 [44–47].

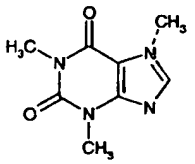
In humans, a homozygous ATM deficiency leads to the disorder known as *Ataxia-telangiectasia* (A-T), which is

characterised by cerebella ataxia, immunodeficiency, oculocutaneous telangiectasia and a predisposition to cancer [47]. Cells derived from A-T patients and from $ATM^{-/-}$ mice display hypersensitivity to ionising radiation, chromosomal instability and defects in the G_1/S, G_2/M checkpoints [48–51]. Drug inhibition studies have confirmed the results from animal and cellular mutation studies.

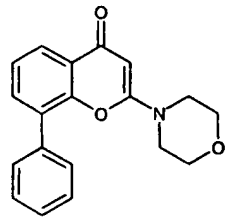
The fungal metabolite wortmannin, for example (compound 3a, Fig. 3), is a potent and selective inhibitor of PIKK family members, including ATM, ATR and DNA-Pkcs [52,53]. Cells that are grown in the presence of wortmannin are sensitised to the killing effects of IR [54–56]. However, such an effect may be put down to the inhibition of DNA-PKcs as well as that of ATM. Certainly, wortmannin has been shown to sensitise an A-T cell-line (AT3BISV, deficient in ATM) and a *scid* murine cell-line (SCF, loss of functioning DNA-PKcs, see below) to X-irradiation but not UV-irradiation, indicating inhibition of DNA-PKcs and ATM, respectively [56]. Another, all be it weaker, inhibitor of ATM and ATR is caffeine (compound 3b, Fig. 3) [57,58]. Caffeine, as well as other xanthine analogues such as pentoxifylline, will, in a similar fashion to wortmannin, sensitise cells to γ- and X-radiation [57,58]. However, this modification is more specific, in that the inhibition of p53 phosphorylation at ser^{15} occurs; an effect directly mediated by ATM and/or ATR, while DNA-PKcs is seen to be resistant to the inhibitory effects of caffeine-like drugs [58].



3a, Wortmannin



3b, Caffeine
[1,3,7-Trimethylxanthine]



3c, LY-294,002
[2-(4-Morpholinyl)-8-phenyl-4H-1-benzopyran-4-one]

Fig. 2. Structures of three PIKK serine/threonine family kinases inhibitors. The natural product wortmannin (3a) is a known inhibitor of ATM, PI3-K and DNA-PKcs. Inhibitory activity is due to covalent binding within the active sites. Caffeine (3b) has been identified as a micromolar inhibitor of ATM and ATR, while having no activity on DNA-PKcs. In contrast, LY294002 (3c) was established as a PI3-K inhibitor that was subsequently shown to inhibit DNA-PKcs, but not ATM. This activity is at low micromolar dose range and involves competitive inhibition of ATP.

A recent study using wortmannin showed that proliferating cells, including tumour cells, are sensitised to a greater degree than normal quiescent cells. It is suggested that the tissue selectivity observed is due to ATM inhibition [59]. This is an important observation because it means that by targeting ATM we can improve the therapeutic ratio for the treatment of tumours over that of normal tissues. A second important finding is that p53-deficient cells are preferentially sensitised to radiation-induced killing by caffeine [60,61]. This suggests that agents which interfere with checkpoint-related proteins may show selectivity for tumour cells bearing intrinsic defects in specific checkpoint pathways.

Another component of the DSB repair mechanism, DNA-PKcs, also presents itself as an interesting target for pharmacological inhibition within cancer biology. This large nuclear kinase (465 kDa protein) works in conjunction with its DNA-binding components termed Ku 70 and 80 [62,63]. It functions, like ATM, in the repair of DNA damage induced by either IR or other strand breaking agents [62–65]. Early studies established the importance of DNA-PK not only in DNA repair as a DSB detection/signalling molecule, but also as a key component in the repair of double strand-breaks created by V(D)J recombination [66,67]. For example, the severe combined immunodeficient mouse (or scid mouse) possesses a truncated DNA-PKcs polypeptide that lacks kinase activity. This results in defective V(D)J recombination (the mice are immuno-compromised), and also an inability to effectively repair IR-induced DSB [68,69]. Like ATM, a DNA-PK inhibitor could have a major impact on enhancing the tumour killing effects of existing irradiation and/or chemotherapeutics that work by creating double strand DNA damage.

To date, no known specific inhibitors of DNA-PK have been identified. Wortmannin will inhibit DNA-PK with an IC_{50} of ~250 nM but, as described, it will also inhibit ATM and PI-3K with IC_{50} values of around 25 and 500 nM, respectively [53]. Another recently described PI-3K inhibitor that inhibits DNA-PK activity is the low molecular weight compound LY294002 [70,71] (compound 3c,

Fig. 3). On SW480 tumour cells, for example, both wortmannin and LY294002 gave sensitisation enhancement ratios of 2.8 and 1.9, respectively (at 10% survival), following irradiation [71]. This is indicative of the fact that LY294002 is a less potent inhibitor of DNA-PK than wortmannin, having an IC_{50} of approximately 1 to 2 μM [53]. Moreover, like wortmannin, LY294002 is non-selective, inhibiting PI-3K at a similar IC_{50}. Although, it does possess more "drug like" qualities than wortmannin, making it more useful for the identification of novel and specific inhibitors to DNA-PKcs. In order to discover novel inhibitors to PIKK family members, modelling based around the published crystal structure of PI-3K active site bound with both wortmannin and LY294002 will be of interest [72]. However, no other crystal structures for PIKK family members have so far been resolved. Moreover, the homology between the active site of the PIKK family members is very low, only between 25 and 30% [72], making an approach using homology modelling difficult.

It is also worth noting that components of the DSB repair pathway may play a role in the modulation of the nucleotide excision pathway. For example, DNA-PK mutant cell-lines (*scid*, V-3 and xrs 6) have been shown to exhibit sensitivity to UV-C irradiation (an NER pathway mechanism) of two- to 2.5-fold. Moreover, wortmannin sensitised parental cells in vivo when combined with UV-C light, but had no effect on DNA-PKcs-deficient *scid* cells. While there is no direct involvement of DNA-PK in NER, a regulatory function of DNA-PK in the NER process may occur [73].

7. Checkpoint proteins

Another group of proteins that represent promising targets in cancer therapy are the cell cycle checkpoint proteins. As discussed above the DSB repair protein ATM plays a central role in controlling cell cycle checkpoint proteins [43]. In proliferating cells an adequate response to DNA damage requires more than repair of the DNA lesion. Upon DNA damage, signal transduction pathways called checkpoints are activated, which delay progression and allow more time for DNA repair to occur. Loss of the G_2/M checkpoint, for example, leads to an increased sensitivity to DNA-damaging agents, suggesting that small molecule inhibitors of the G_2/M checkpoint response may be useful for targeting in cancer therapy, in combination with radio- and chemosensitising agents [74,75]. Moreover, a disruption of cell cycle checkpoints in cancer cells may improve the therapeutic index [75,76]. This is suggested by the findings that pharmacological inhibition of G_2 checkpoints can increase sensitivity to chemotherapy in G_1 checkpoint-deficient cells, whereas cells with normal checkpoints may take refuge in G_1 [76].

Two key proteins have been identified in cell cycle checkpoints: Chk1 and Chk2. Both are serine/threonine kinases, which are structurally unrelated but share some overlapping substrate specificities [61]. These kinases are involved in the downstream signalling to the cell cycle G_2/M and G_1/S phases, respectively [77a]. Compounds that inhibit the G_2 checkpoint by targeting Chk1/Chk2 may be valuable in cancer therapy to enhance the effectiveness of DNA-damaging agents in tumours with defective G_1 DNA damage checkpoint, such as those with mutated p53 [77b–79]. Two such inhibitors, caffeine and UCN-01 (7-hydroxystaurosporine) (Fig. 4), have been shown to abrogate the G_2 checkpoint in tumour cells by inhibiting ATM and Chk1, respectively [61,80]. Such inhibitors, when used to treat cells that have lost the p53-dependent checkpoint controls, result in the selective sensitisation of cancer cells to conventional genotoxic therapies over that of normal cells [80–82].

Few G_2 checkpoint inhibitors are currently known, and while caffeine and caffeine analogues inhibit ATM and ATR protein kinases (see above), they are not considered to be drug candidates. UCN-01, an analogue of staurosporine, is being evaluated in Phase I clinical trials for the

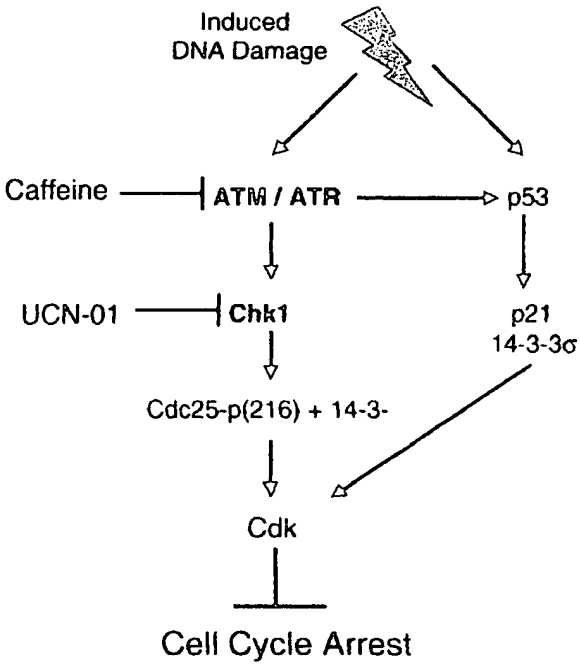


Fig. 4. Model showing the points of G_2 checkpoint inhibition of caffeine and 7-hydroxystaurosporine (UCN-01). DNA damage leads to two parallel signal transduction pathways that prevent premature progression from G_2 into mitosis. Chk1 phosphorylates Cdc25 at serine 216, leading to its sequestration into cytoplasm, thus preventing the dephosphorylation of Cdk1 causing temporary cell cycle arrest. Both caffeine and UCN-01 abrogate G_2 cell cycle arrest by inhibiting the upstream ATM and downstream Chk1 activities, respectively. However, inhibition of cell cycle Chk1 dependent control will lead to loss of checkpoint integrity only if the p53-dependent signalling pathway has been compromised.

treatment of cancer [83]; unfortunately, it has been shown in vitro to inhibit several other protein kinases, making selectivity an issue [84]. Recently, debromohymenialdisine (DBH) has been identified as a selective Chk1 and Chk2 inhibitor, suggesting the possibility of controlling the cell cycle independent of upstream regulators, such as ATM [85].

8. O^6-Alkylguanine-DNA alkytransferase

Finally, I would like to briefly discuss O^6-alkylguanine-DNA alkytransferase (AGT), since this enzyme has been linked to increased resistance of cells to alkylating agents [86], and as such has important clinical implications. AGT acts to eliminate alkyl groups that have been introduced onto the O^6-position of guanine [87] by transferring them to a cysteine residue within its own active centre. The result of this repair mechanism is the restoration of guanine in the DNA and the inactivation of AGT [87]. O^6-Benzylguanine has been shown to be a potent inhibitor of AGT activity, and its use has been demonstrated in cell-lines and isolated lymphocytes from patients with chronic lymphocytic leukaemia, to enhance the cytotoxicity of alkylating agents including nitrosoureas, such as 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU), and certain monofunctional alkylating agents [87,88]. Phase I trials of O^6-benzylguanine have already been carried out with the aim of modulating AGT activity and thereby circumventing drug resistance in the clinic [89].

9. Concluding remarks

There is substantial evidence that the majority of inhibitors that target the central "controlling" proteins in DNA repair, such as PARP, ATM, DNA-PKcs and CHK1/CHK2, will dramatically improve the therapeutic benefits of the existing genotoxic therapies that currently dominate the cancer treatment markets. Moreover, the inhibition of some of these processes involved in DNA repair and cell cycling may in their own right prove to have direct anti-cancer activity. This will only become apparent once potent and selective inhibitors become available for pharmacological studies over the next few years. Nevertheless, there still remain many unanswered questions on the mechanisms whereby the damage-signalling kinases, ATM, DNA-PKcs for example, are activated and how they select and signal to the DNA repair processes.

Acknowledgements

I would like to thank my colleagues, Dr. R. Hather, Dr. B. Ward and Dr. G.C.M. Smith, for their help in preparing the manuscript.

References

[1] Medical and Healthcare Market Place Guide; Cancer, in: Time Series, Dorlands Directories, 1999.
[2] P.A. Kiberstis, Cancer therapy on target, Science 292 (2001) 399–401.
[3] D. Ferber, A new way to combat therapy side effects, Science 285 (1999) 1651–1653.
[4] E.C. Friedberg, G.C. Walker, W. Siedo, DNA Repair and Mutagenesis, ASM Press, Washington, DC, 1995.
[5] B.A.J. Ponder, Cancer genetics, Nature 411 (2001) 336–341.
[6] J. Cadet, M. Berger, T. Douki, J.L. Ravanat, Oxidative damage to DNA: formation, measurement and biological significance, Rev. Physiol. Biochem. Pharmacol. 131 (1997) 239–247.
[7] T. Finkel, N.J. Holbrook, Oxidants, oxidative stress and the biology of aging, Nature 408 (2000) 239–247.
[8] J.H.J. Hoeijmakers, Genome maintenance mechanisms for preventing cancer, Nature 411 (2001) 366–374.
[9] J.A. Lippke, L.K. Gordon, D.E. Brash, W.A. Haseltine, Distribution of UV light-induced damage in a defined sequence of human DNA: detection of alkaline-sensitive lesions at pyrimidine nucleoside–cysteine sequences, Proc. Natl. Acad. Sci. USA 78 (1981) 3388–3392.
[10] E.C. Friedberg, G.C. Walker, W. Siede, DNA damage, in: E.C. Friedberg, G.C. Walker, W. Siede (Eds.), DNA Repair and Mutagenesis, ASM Press, Washington, DC, 1995, pp. 1–47.
[11] D.P. Batty, R.D. Wood, Damage recognition in nucleotide excision repair of DNA, Gene 241 (2000) 193–204.
[12] D. Bootsma, K.H. Kraemer, J. Cleaver, J.H.J. Hoeijmakers, in: C.R. Scriver, A.L. Beaudet, W.S. Sly, D. Valle (Eds.), The Metabolic and Molecular Basis of Inherited Disease, Vol. 1, McGraw-Hill, New York, 2001, pp. 677–703.
[13] A.R. Lehmann, The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases, Genes Dev. 15 (2001) 15–23.
[14] S.S. Parik, C.D. Mol, J.A. Tainer, Base excision repair enzymes family portrait integrating the structure and chemistry of an entire DNA repair pathway, Structure 5 (12) (1997) 1543–1550.
[15] G. De Murcia, S. Shall, From DNA Damage and Stress Signalling to All Death Poly ADP-ribosylation Reactions, Oxford University Press, 2000.
[16] J.M. De Murcia, C. Niedergang, C. Trucco, M. Ricoul, B. Dutrillaux, M. Mark, F.J. Oliver, M. Masson, A. Dierich, M. leMeur, C. Walztinger, P. Chambon, G. de Murcia, Requirement of poly(ADP-ribose) polymerase in recovery from DNA damage in mice and in cells, Proc. Natl. Acad. Sci. 94 (1997) 7303–7307.
[17] F.J. Oliver, G. de la Rubia, V. Rolli, M.C. Ruiz-Ruiz, G. de Murcia, J.M. Murcia, Importance of poly(ADP-ribose) polymerase and its cleavage in apoptosis, lesson from an uncleavable mutant, J. Biol. Chem. 273 (1998) 33533–33539.
[18] Z. Herceg, Z.Q. Wang, Functions of poly(ADP-ribose) polymerase (PARP) in DNA repair, genomic integrity and cell death, Mutat. Res. 477 (2000) 97–110.
[19] M. Masutani, T. Nozaki, E. Nishiyama, T. Shimokawa, Y. Tachi, H. Suzuki, H. Nakagama, K. Wakabayashi, T. Sugimura, Function of poly(ADP-ribose) polymerase in response to DNA damage: gene-disruption study in mice, Mol. Cell. Biochem. 193 (1999) 149–152.
[20] N.J. Curtin, B.T. Golding, R.J. Griffin, D.R. Newell, M.J. Roberts, S. Srinivasan, A.W. White, New poly(ADP-ribose) polymerase inhibitors for chemo- and radiotherapy of cancer, in: G. de Murcia, S. Shall (Eds.), From DNA Damage and Stress Signalling to Cell Death, Poly ADP-ribosylation Reactions, Oxford University Press, Oxford, 2000, pp. 177–198.
[21] M.R. Purnell, W.J.D. Whish, Novel inhibitions of poly(ADP-ribose) synthetase, Biochem. J. 185 (1980) 775–779.
[22] A. Ruf, J.M. de Murcia, G. de Murcia, G.E. Schulz, Structure of the

catalytic fragment of poly(ADP-ribose) polymerase from chicken, Proc. Natl. Acad. Sci. USA 93 (1996) 7481–7485.
[23] R.J. Griffin, S. Srinivasan, K. Bowman, A.H. Calvert, N.J. Curtin, D.R. Newell, L.C. Pemberton, B.T. Golding, Resistance-modifying agents 5′ synthesis and biological properties of quinazolinone inhibitors of the DNA repair enzyme poly(ADP-ribose)polymerase (PARP), J. Med. Chem. 41 (1998) 5247–5256.
[24] E. Perkins, D. Sun, A. Nguyen, S. Tulac, M. Francesco, H. Tavana, H. Nguyen, S. Tugenndreich, P. Barthmaier, J. Couto, E. Yeh, S. Thode, K. Jamagin, A. Jain, D. Morgans, T. Melese, Novel inhibitors of poly(ADP-ribose) polymerase/PARP1 and PARP2 identified using a cell-based screen in yeast, Cancer Res. 61 (2001) 4175–4183.
[25] M.J. Suto, W.R. Turner, C.M. Arundel-Suto, L.M. Werbel, J.S. Sebolt-Leopold, Dihydroisoquinolines: the design and synthesis of a new series of potent inhibitors of poly(ADP-ribose)polymerase, Anticancer Drug Des. 7 (1991) 107–112.
[26] E.B. Affar, M. Germain, G.G. Poirier, Role of poly(ADP-ribose) polymerase in cell death, in: G. de Murcia, S. Shall (Eds.), From DNA Damage and Stress Signalling to Cell Death, Poly ADP-ribosylation Reactions, Oxford University Press, Oxford, 2000, pp. 125–150.
[27] A. Schlicker, P. Peschke, A. Burkle, E.W. Hahn, J.H. Kim, 4-Amino-1,8-naphthalimide: a novel inhibitor of poly(ADP-ribose)polymerase and radiation sensitizer, Int. J. Radiat. Biol. 75 (1) (1999) 91–100.
[28] C.A. Delaney, L.-Z. Wang, S. Kyle, A.W. White, A.H. Calvert, N.J. Curtin, B.W. Durkacz, Z. Hostonisky, D.R. Newell, Potentiation of temozolomide and topotecan growth inhibition and cytotoxicity by novel poly(adenosine diphosphoribose)polymerase inhibitions in a panel of human tumor cell lines, Clin. Cancer Res. 6 (2000) 2860–2867.
[29] T. Kato, Y. Suzumura, M. Fukushina, Enhancement of bleomycin activity by 3-aminobenzamide, a poly(ADP-ribose) synthesis inhibitor, in vitro and in vivo, Anticancer Res. 8 (1988) 239–244.
[30] G. Chen, Q.C. Pan, Potentiation of the antitumour activity of cisplatin in mice by 3-aminobenzamide, Cancer Chemother. Pharmacol. 22 (1988) 303–309.
[31] C. Petrou, D. Mourelatos, E. Miolou, J. Dozi-Vassiliads, P. Catsoulacos, Effects of alkylating antineoplastics alone or in combination with 3-aminobenzamide in genotoxicity, antitumour activity and NAD levels in human lymphocytes in vitro and Ehrlich ascites tumour cells in vivo, Teratog. Carcinog. Mutagen. 10 (1990) 321–328.
[32] F. Darraudi, T.S.B. Zwanenberg, A.T. Natarajan, O. Driesseu, A. van Langevelde, Reduced tumour progression in vivo by inhibitor of poly(ADP-ribose) synthetase (3-aminobenzamide) in combination with X-rays or the cytostatic drug DTIC, in: M.K. Jackson, E.L. Jackson (Eds.), ADP-ribose Transfer Reactions: Mechanisms and Biological Significance, Springer, New York, 1989, pp. 390–410.
[33] W.R. Leopold, J.S. Sebolt-Leopold, Chemical approaches to improved radiotherapy, in: F.A. Valeriote, T.H. Corbett, L.H. Baker (Eds.), Cytotoxic Anti-cancer Drugs: Models and Concepts For Drug Discovery and Development, Kluwer, Boston, MA, 1990.
[34] J.C. Ame, V. Rolli, V. Schreiber, C. Niedergang, F. Apiou, P. Decker, S. Muller, T. Hoger, J.M. de Murcia, G. de Murcia, PARP-2, a novel mammalian DNA damage-dependent poly(ADP-ribose) polymerase, J. Biol. Chem. 274 (1999) 17860–17868.
[35] S. Smith, The world according to PARP, TIPS 26 (3) (2001) 174–179.
[36] M. Tsutsumi, M. Masutani, T. Nozaki, O. Kusuoka, T. Tsujiuchi, H. Nakagama, H. Suzuki, Y. Konishi, T. Sugimura, Increased susceptibility of poly(ADP-ribose) polymerase-1 knockout mice to nitrosamine carcinogenicity, Carcinogenesis 22 (1) (2001) 1–3.
[37] J. Wesierska-Gadek, A. Bugajska-Schrette, C. Corni, ADP-ribosylation of p53 tumor suppressor protein: mutant but not wild-type p53 is modified, J. Cell. Biochem. 62 (1996) 90–101.
[38] M. Malanga, J.M. Pleschke, H.F. Kleozkowska, F.R. Althaus, Poly(ADP-ribose) binds to specific domains of p53 and alters its DNA binding functions, J. Biol. Chem. 273 (1998) 11839–11843.
[39] C. Conde, M. Mark, F.J. Oliver, A. Huber, G. de Murcia, J.M. de Murcia, Loss of poly(ADP-ribose) polymerase-1 causes increased tumor latency in p53 deficient mice, EMBO J. 20 (13) (2001) 3535–3543.
[40] B.B. Zhou, S.J. Elledge, The DNA damage response: putting checkpoints in perspective, Nature 408 (2000) 433–439.
[41] K.K. Khanna, S.P. Jackson, DNA double strand breaks: signalling, repair and the cancer connection, Nature Genet. 27 (2001) 247–254.
[42] P.A. Jeggo, DNA-PK: at the cross-roads of biochemistry and genetics, Mutat. Res. 384 (1997) 1–14.
[43] D. Durocher, S.P. Jackson, DNA-PK, ATM and ATR as sensors of DNA damage: variations on a theme?, Curr. Opin. Cell Biol. 13 (2001) 225–231.
[44] C.E. Canman, D.S. Lim, K.A. Cimprich, Y. Taya, K. Tamai, K. Sakaguchi, E. Appella, M.B. Kastan, J. Sipiciano, Activation of the ATM kinase by ionising radiation and phosphorylation of p53, Science 281 (1998) 1677–1679.
[45] R. Khosravi, R. Maya, T. Gottlieb, M. Oren, Y. Shiloh, D. Shkedy, Rapid ATM-dependent phosphorylation of MDM2 recedes p53 accumulation in response to DNA damage, Proc. Natl. Acad. Sci. USA 96 (1999) 14973–14977.
[46] D. Cortez, Y. Wang, J. Qin, S.J. Elledge, Requirement of ATM-dependent phosphorylation of BRCA1 in the DNA damage response to double-strand breaks, Science 286 (1999) 1162–1166.
[47] B.-B.S. Zhou, S.J. Elledge, The DNA damage response: putting checkpoints in perspective, Nature 408 (2000) 433–439.
[48] G. Rotman, Y. Shiloh, ATM: from gene to function, Hum. Mol. Genet. 7 (1998) 1555–1563.
[49] R.B. Painter, B.R. Young, Radiosensitivity in ataxia-telangiectasia: a new explanation, Proc. Natl. Acad. Sci. USA 77 (1980) 7315–7317.
[50] C. Barlow, S. Hirotsume, R. Paylor, M. Liyanage, M. Eckhaus, F. Collins, Y. Shiloh, J.N. Crawley, T. Ried, D. Tagle, A. Wynshaw-Boris, ATM deficient mice a paradigm of ataxia-telangiectasia, Cell 86 (1996) 159–171.
[51] M.B. Kastan, Q. Zhan, W.S. el-Deiry, F. Carrier, T. Jacks, W.W. Walsh, B.S. Plunkett, B. Vogelstein, A.T. Fornace Jr., A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia, Cell 71 (1992) 587–597.
[52] J.N. Sarkaria, R.S. Tibbetts, E.C. Busby, A.P. Kennedy, D.E. Hill, R.T. Abraham, Inhibition of phosphoinositide 3-kinase related kinases by the radiosensitivity agent wortmannin, Cancer Res. 58 (1998) 4375–4382.
[53] R.A. Izzard, S.P. Jackson, G.C.M. Smith, Competitive and non competitive inhibition of the DNA-dependent protein kinase, Cancer Res. 59 (1999) 2581–2586.
[54] B.D. Price, M.B. Youmell, The phosphatidylinositol 3-kinase inhibitor wortmannin sensitises murine fibroblasts and human tumor cells to radiation and blocks induction of 53 following DNA damage, Cancer Res. 56 (1995) 246–250.
[55] S. Boulton, S. Kyle, L. Yalcintepe, B.W. Durkacz, Wortmannin is a potent inhibitor of DNA double strand break but not single strand break repair in Chinese hamster ovary cells, Carcinogenesis (Lond.) 17 (1997) 2285–2290.
[56] Y. Hosoi, H. Miyachi, Y. Matsumoto, H. Ikehata, J. Komura, J. Ishii, H.J. Zhao, M. Yoshida, Y. Takai, S. Yamada, N. Suzuki, T. Ono, A phosphatidylinositol 3-kinase inhibitor wortmannin induces radioresistant DNA synthesis and sensitises cells to bleomycin and ionising radiation, Int. J. Cancer 78 (1998) 642–647.
[57] L.J. Tolmach, R.W. Jones, P.M. Busse, The action of caffeine on X-irradiated HeLa cells. 1. Delayed inhibition of DNA synthesis, Radiat. Res. 71 (1977) 653–665.
[58] J.N. Sarkaria, E.C. Busby, R.S. Tibbetts, P. Roos, Y. Taya, L.M. Kamitz, R.T. Abraham, Inhibition of ATM and ATR kinase activity by the radiosensitizing agent, caffeine, Cancer Res. 59 (1999) 4375–4382.

[59] Y.Q. Shi, H. Blattmann, N.E.A. Crompton, Wortmannin selectively enhances radiation-induced apoptosis in proliferative but not quiescent cells, Int. J. Radiat. Oncol. Biol. Phys. 49 (2) (2001) 421–425.
[60] K.J. Russell, L.W. Wiens, G.W. Demers, D.A. Galloway, S.E. Plon, M. Groudine, Abrogation of the G2 checkpoint results in differential radiosensitization of G1 checkpoint-deficient and G1 checkpoint-competent cells, Cancer Res. 55 (1995) 1639–1642.
[61] S.N. Powell, J.S. DeFrank, P. Connell, M. Eogan, F. Preffer, D. Dombkowski, W. Tang, S. Friend, Differential sensitivity of p53 (−/−) and p53 (+/+) cells to caffeine-induced radiosensitization and override of G2 delay, Cancer Res. 55 (1995) 1643–1648.
[62] S.P. Jackson, DNA damage detection by DNA dependent protein kinase and related enzymes, Cancer Surv. 28 (1996) 261–279.
[63] K.K. Leuther, O. Hammarsten, R.D. Kornberg, G. Chu, Structure of DNA-dependent protein kinase: implications for its regulation by DNA, EMBO J. 18 (5) (1999) 1114–1123.
[64] T.M. Gottlieb, S.P. Jackson, The DNA-dependent protein kinase: requirement for DNA ends and associated with Ku antigen, Cell 72 (1993) 131–142.
[65] M.R. Lieber, U. Grawunder, X.T. Wu, M. Yaneva, Tying loose ends: roles of Ku and DNA-dependent protein kinase in the repair of double strand breaks, Curr. Opin. Genet. Dev. 7 (1997) 96–104.
[66] T. Blunt, N.J. Finnie, G.E. Taccioli, G.C. Smith, J. Demengeot, T.M. Gottlieb, R. Mizuta, A.J. Varghese, F.W. Alt, P.A. Jeggo, Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine scid mutation, Cell 80 (1995) 813–823.
[67] Y. Gao, J. Chaudhuri, C. Zhu, L. Davidson, D.T. Weaver, F.W. Alt, A targeted DNA-PKcs — null mutation reveals DNA-PK-independent functions for KU in V(D)J recombination, Immunity 9 (1998) 367–376.
[68] J.S. Danska, D.P. Holland, S. Mariathasan, K.M. Williams, G.J. Guidos, Biochemical and genetic defects in the DNA-dependent protein kinase in murine scid lymphocytes, Mol. Cell. Biol. 16 (1996) 5507–5517.
[69] R. Araki, A. Fujimori, K. Hamatani, K. Mita, T. Saito, M. Mori, R. Fukumura, M. Morimyo, M. Muto, M. Itoh, K. Tatsumi, M. Abe, Nonsense mutation at tyr-4046 in the DNA-dependent protein kinase catalytic submit of severe combined immune deficiency mice, Proc. Natl. Acad. Sci. USA 94 (1997) 2438–2443.
[70] C.J. Vlahos, W.F. Matter, K.Y. Hui, R.F. Brown, A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY294002), J. Biol. Chem. 269 (1994) 5242–5248.
[71] K.F. Rosenzweig, M.B. Youmell, S.T. Palayoor, B.D. Price, Radiosensitization of human tumor cells by the phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G_2-M delay, Clin. Cancer Res. 3 (1997) 1149–1156.
[72] E.H. Walker, M.E. Pacold, O. Perisic, L. Stephens, P.T. Hawkins, M.P. Wymann, R.L. Williams, Structural determinants of phosphoinositide 3-kinase inhibition by wortmannin, LY294002, quercetin, myricetin and staurosporine, Mol. Cell. 6 (2000) 909–919.
[73] C. Muller, P. Calsou, P. Frit, C. Cayrol, T. Carter, B. Salles, UV sensitivity and impaired nucleotide excision repair in DNA-dependent protein kinase mutant cells, Nucleic Acids Res. 26 (6) (1998) 1382–1389.
[74] T. Enoch, C. Norbury, Cellular response to DNA damage: cell-cycle checkpoints, apoptosis and the roles of p53 and ATM, TiBS 20 (1995) 427–431.
[75] T.A. Weinert, DNA damage and checkpoint pathways: molecular anatomy and interactions with repair, Cell 94 (1998) 555–558.
[76] L.H. Hartwell, M.B. Kastan, Cell cycle control and cancer, Science 266 (1994) 1821–1828.
[77] (a) M.J. O'Connell, N.C. Walworth, A.M. Carr, The G2-phase DNA-damage checkpoint, Trends Cell Biol. 10 (2000) 296–303; (b) D.E. Fisher, Apoptosis in cancer therapy: crossing the threshold, Cell 78 (1994) 539–542.
[78] M.V. Blagosklonny, A.B. Pardee, Exploiting cancer cell cycling for selective protection of normal cells, Cancer Res. 61 (2001) 4301–4305.
[79] P. Nurse, Checkpoint pathways come of age, Cell 91 (1997) 865–867.
[80] E.C. Busby, D.F. Leistritz, R.T. Abraham, L.M. Karnitz, J.N. Sarkania, The radiosensitizing agent 7-hydroxystaurosporine (UCN-01) inhibits the DNA damage checkpoint kinase hChk1, Cancer Res. 60 (2000) 2108–2112.
[81] K.J. Russell, L.W. Wiens, G.W. Demers, D.A. Galloway, S.E. Plon, M.G. Groudine, Abrogation of the G_2 checkpoint-competent cells, Cancer Res. 55 (1995) 1639–1642.
[82] S.-L. Yao, A.J. Akhtar, K.A. McKenna, G.C. Bedi, D. Sidransky, M. Mabry, R. Ravi, M.L Collection, R.J. Jones, S.J. Sharkis, E.J. Fuchs, A. Bedi, Selective radiosensitization of p53-deficient cells by caffeine-mediated activation of $p34^{cdc2}$ kinase, Nat. Med. 2 (1996) 1140–1143.
[83] E.A. Sausville, S.G. Arbuck, R. Messmann, D. Headlee, K.S. Bauer, R.M. Lush, A. Murgo, W.D. Figg, T. Lahusen, S. Jaken, X. Jing, M. Roberge, E. Fuse, T. Kuwabara, A.M. Senderowicz, Phase I trial of 72-hour continuous infusion of UCN-01 in patients with refractory neoplasms, J. Clin. Oncol. 19 (2001) 2319–2330.
[84] K. Kawakami, H. Futami, J. Takahara, K. Yamaguchi, UCN-01, 7-hydroxy-staurosporine, inhibits kinase activity of cyclin-dependent kinases and reduces the phosphorylation of the retinoblastoma susceptibility gene product in A549 human cancer cell lines, Biochem. Biophys. Res. Commun. 219 (1996) 778–783.
[85] D. Curman, B. Cinel, D.E. Williams, N. Rundle, W.D. Block, A.A. Goodarzi, J.R. Hutchins, P.R. Clarke, B.B. Zhou, S.P. Lees-Miller, R.J. Andersen, M. Roberge, Inhibition of the G2 DNA damage checkpoint and of protein kinases Chk1 and Chk2 by the marine sponge alkaloid debromohymenialdisine, J. Biol. Chem. 276 (2001) 17914–17919.
[86] M.E. Dolan, R.C. Moschul, A.E. Pegg, Depletion of mammalian O^6-alkylguanine-DNA alkyltransferase activity by O^6-benzylguanine provides a means to evaluate the role of this protein in protection against carcinogenic and therapeutic alkylating agents, Proc. Natl. Acad. Sci. USA 87 (1990) 5368–5372.
[87] A. Sancer, DNA repair in humans, Annu. Rev. Genet. 29 (1995) 69–105.
[88] M.R. Muller, J. Thomale, C. Lensing, M.F. Rajewsky, S. Seeber, Chemosensitisation to alkylating agents by pentoxifylline, O^6-benzylguanine, and ethacrynic acid in haematological malignancies, Anticancer Res. 13 (1993) 2155–2160.
[89] H.S. Friedman, D.M. Kokkinakis, J. Pluda, A.H. Friedman, I. Cokgor, M.M. Haglund, D.M. Ashley, J. Rich, M.E. Dolan, A.E. Pegg, R.C. Moschel, R.E. M^{c}Lendon, T. Kerby, J.E. Herndon, D.D. Bigner, S.C. Schold Jr., Phase I trial of O^6-benzylguanine for patients undergoing surgery for malignant glioma, J. Clin. Oncol. 16 (1998) 3570–3575.